An Experimental Study on the Effect of Low-Intensity Ultrasound on Developing Mouse Embryos[1)2)]

By

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(With 6 Text-figures and 2 Tables)

The Doppler effect of ultrasound waves has been recently applied to a diagnostic purpose in obstetrics on early detection of fetal life and determination of intrauterine death. Some clinical investigators have not demonstrated any untoward or toxic effect of diagnostic ultrasound on any type of tissue with the power levels which are currently used (Thompson 1968, Barton 1968). Up-to-date experimental results indicated by a few workers have differed according to different conditions (Fritz-Niggli *et al.* 1950, Selman *et al.* 1953, 1964, Holmes *et al.*, 1962, Andrew 1964, Pourhadi *et al.* 1968, Takeuchi *et al.* 1970). No evidence which is able to assure the safety, with confidence, on the normal development of embryos exposed to ultrasound waves has been established as yet, although diagnostic energy was low intensity.

The present authors have projected embryological and cytogenetic studies on teratogenic action of ultrasound waves on mammalian embryos. This report indicates primary data in an animal experiment on effects of low-intensity of ultrasound waves on developing mouse embryos.

Materials and Methods

The animals used in the present study were DHS mice from a inbred strain maintained in our laboratory for more than 45 generations. Estrus females aged about 60 to 90 days were caged overnight with males. The onset of pregnancy was indicated by finding vaginal plug. This day was designated as the first day of gestation. The females with plugs were separated in three groups as shown in Table 1. Each animal of the first group, on the 9th day of gestation, was anesthetized with 0.03 ml of nembutal sodium (Abott Laboratory),

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injected intraperitoneally, and bound on the plate. Then they were exposed to ultrasound waves with the frequency of 2.25 MHz and the intensity of 40 mW/cm² by Doppler Fetal Detector (Nippon Electric Co. Ltd., Model 101), for 5 hours. For direct application of the probe, glycerin was used as a coupling medium. Mice of the second group, namely bound control, were prepared under the same manipulation, without the radiation of ultrasound waves in the first group. The third group was served as untreated control. Thus two control groups were set up in the present experiment. All mice of ultrasound and control groups were sacrificed on the 18th day of gestation and developmental condition of the fetuses were examined. The fetuses were recorded as being alive or dead in each uterus. All living fetuses were examined for the occurrence of gross external malformations under a dissecting microscope. The resorption sites and placental remnants were regarded as dead fetuses.

Results

Each pregnancy rate in females of three groups was about 70 per cent. Mean values of implantations in them also were 7.6 in ultrasound group, and 7.2 in both bound and untreated control groups.

Table 1. Teratogenic effect of ultrasound on mouse embryos on the 9th day of gestation

Treatment	No. of pregnancy	No. of living fetus	malformed fetus	
			no. (%)	type*
Ultrasound	21	128	8 (6.3)	Ex, Oe, Ag, Abg, Hp, Md
Bound control	25	163	5 (3.1)	Im, Cp, Abg, Hp, Ag
Untreated control	25	158	3 (2.0)	Ag, Hp

Values in parentheses represent percentage for number of living fetus. The result of "t" test for malformed fetus was insignificant between untreated control group and ultrasound group.

* Ex: Exencephaly, Oe: Open eyelid, Ag: Agnathia, Im: Incomplete mandible, Cp: Cleft palate, Hp: Hindfoot polydactyly, Md: Macrodactyly, Abg: Abnormal genitalia

As shown in Table 1, eight types of fetal malformations were found in the present observation: exencephaly, open eyelid, agnathia, incomplete mandible, cleft palate, hindfoot polydactyly, macrodactyly and abnormal genitalia. Some representative malformations were shown in Text-figures 1–6. Only one abnormal fetus with exencephaly, open eyelid and hindfoot polydactyly was obtained from a mother exposed to ultrasound waves. The percentages of fetuses with those anomalies to living fetuses examined were 6.3 in ultrasound group, 3.1 in bound control group and 2.0 in untreated control group. The difference in the total

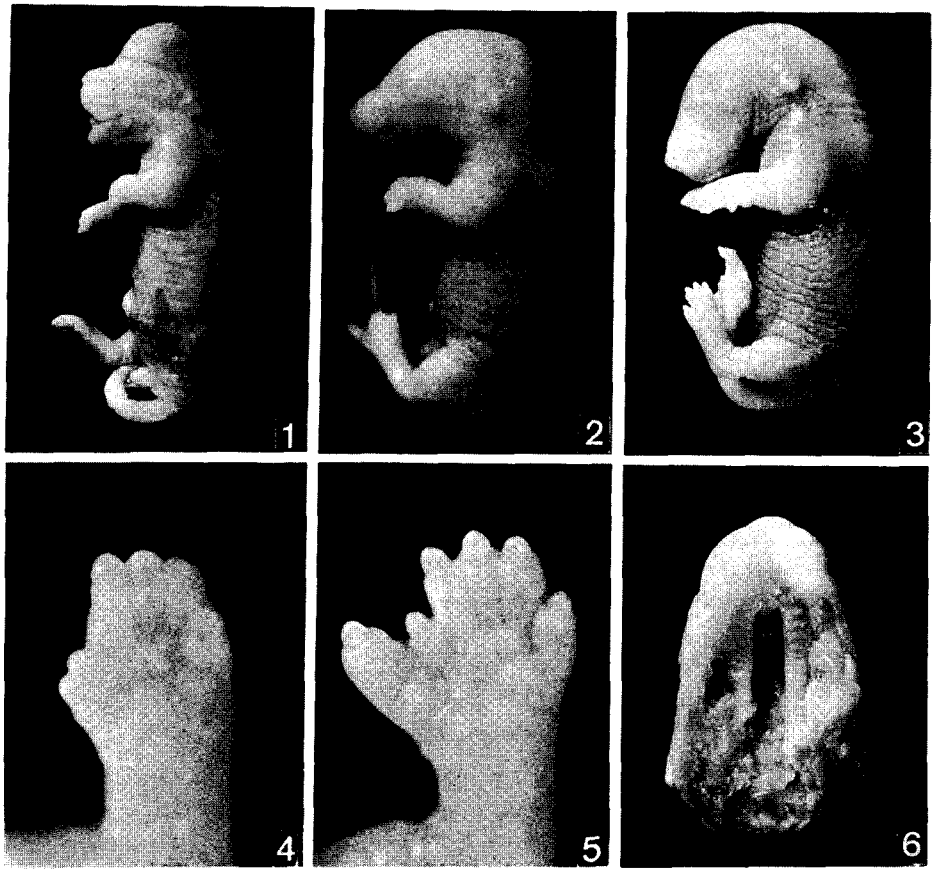

Fig. 1. A malformed fetus with exencephaly, open eyelid and left hindfoot polydactyly obtained from a mother mouse exposed to ultrasound waves (frequency; 2.25 MHz, acoustic intensity; 40 mW/cm^2) on the 9th day of gestation.

Fig. 2. A fetus with incomplete mandible, from a female radiated ultrasound.

Fig. 3. An abnormal fetus with aganthia, from an untreated mouse.

Fig. 4. Left hindfoot polydactyly from the same fetus shown in Fig. 1.

Fig. 5. Left hindfoot polydactyly and macrodactyly in a fetus obtained from a mother mouse exposed to ultrasound.

Fig. 6. Cleft palate obtained from a mouse fetus of bound group.

number of them was statistically insignificant between the ultrasound and two control animals.

Mean values of body weights of living fetuses were 0.78 gr. in ultrasound group, 0.79 gr. in bound group and 0.82 gr. in untreated group, on the 18th day of

gestation (Table 2). In addition, the distribution of those measured individually also was roughly identical in three groups.

Table 2. Lethal effect of ultrasound on mouse embryos on the 9th day of gestation

Treatment	No of. pregnancy	No. of implantation (mean 1. size)	Living fetus		No. of dead fetus (%)
			No. (%)	body weight (g)	
Ultrasound	21	160 (7.6)	128 (80.0)	0.78	32*(20.0)
Bound control	25	181 (7.2)	163 (90.1)	0.79	18 (9.9)
Untreated control	25	179 (7.2)	158 (88.3)	0.82	21 (11.7)

Values in parentheses represent percentage for number of implantations.

* Difference between untreated control and experimental values was significant at 5 per cent level, according to "t" test.

The frequency of fetal death involved embryonal death, in ultrasound-exposed mice, reached 20.0 per cent as compared with 9.9 per cent in bound control and 11.7 per cent in untreated control mice (Table 2). Statistical analyses failed in detecting any significant difference in frequency between bound and untreated control groups, while it was signfiicant between ultrasound and untreated control groups ($P<0.05$).

Discussion

A few investigators have reported that ultrasound waves employing the power and the technique in their experiments had not teratogenic effects on developing embyors of animals such as rats (Takeuchi *et al.* 1970), rabbits (Holmes *et al.* 1962) and forgs (Andrew 1964). Whereas Fritz-Niggli *et al.* (1950) and Selman *et al.* (1953) indicated that the treatment with ultrasound waves of eggs of *Drosophila melanogaster* induced abnormal embryonic development, and that the susceptibility of the eggs on ultrasound waves changed remarkably at the various stages in development. In a recent study on teratogenic actions of ultrasound waves on amphibian eggs (*Triturus helveticus* and *Triturus alpestris*), Pourhadi *et al.* (1968) have found maldeveloped larvae with various anomalies such as cyphosis, lordosis, cyclopia, synophthalmia, anophthalmia, anterior, posterior or mixed duplications. It seems likely that the different findings described by the above investigators are due to the different conditions with the animal species or the frequency and the acoustic intensity of ultrasound waves employed in their experiments.

In the present experiment, eight types of malformations in mouse fetuses were observed, and the frequency in occurrence of the abnormal fetuses with those was low. However, it may be a noteworthy fact that an exencephalous fetus was

inspected in a mouse exposed to ultrasound waves, although the fetus was only one (Fig. 1). A continual investigation during the last 5 years has been carrying out about fetal malformations occurred spontaneously in mice of inbred strains maintained in our laboratory. So far as the data collected from the investigation, no exencephalous fetus has been found among 1797 living fetuses derived from 272 untreated mother mcie of DHS strain used in the present experiment. On the other hand the other types except exencephaly of such defects shown in the present results (Table 1) frequently occurred spontaneously. The details on the spontaneous malformations in these investigations will be separately reported. The exencephaly induced experimentally also has been demonstrated in many teratological studies with various extrinsic agents, such as x-rays (Russell 1950), anoxia (Ingalls *et al.* 1950), trypan blue (Hamburgh 1954), hypervitaminosis A (Murakami *et al.* 1965), vincristine (DeMyer 1965), colchicine (Shoji 1968a) and nervenruh forte (Shoji 1968b). They have described that the induction of the exencephaly might be associated with the action of those extrinsic agents used in their studies respectively.

Takeuchi *et al.* (1970), working on effects of ultrasound waves with frequency of 2.25 MHz and acoustic intensity of 150 mW/cm^2 on embryonal ova of rats, reported that the mean body weight and the mortality of fetuses in uteri were not significantly different in both experimental and control rats. In the present results, the mean value of body weight of mouse fetuses was identical in both experimental and control groups, while the difference of the fetal mortality between ultrasound group and untreated group indicated statistical significance.

The present data, therefore, seems to suggest that the maternal exposure to ultrasound waves increases in frequency of maldevelopment or intrauterine death in mouse embryos.

Summary

Effects of low-intensity ultrasound on mouse embryos of DHS strain were investigated with special regard to the fetal malformation, growth and death. On the 9th day of gestation, pregnant females were radiated with a frequency of 2.25 MHz and an acoustic intensity of 40 mW/cm^2 of ultrasound waves by Doppler Fetal Detector for 5 hours. Fetuses with external abnormalities occurred in both experimental and control animals. The difference in frequency of those abnormalities between experimental and control groups was not statistically significant. But one fetus with exencephaly was observed only in ultrasound-group, which was never spontaneously found in mice of this strain. The incidence of dead fetuses was increased by ultrasound radiation. The fetal mortality was significantly different between experimental and untreated control groups. No growth-inhibiting effect on the fetuses was noticed in the data of body weight.

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